Mapping of non-recombining regions via molecular markers

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ABSTRACT

The lack of recombination in some genomic regions represents a serious obstacle in mapping studies. In this review, we describe methods that are currently used to overcome this problem. Main attention is given to the comparison of methods that are based on the principle of radiation hybrid mapping. We also discuss a strategy called HAPPY mapping (mapping based on the analysis of approximately HAPloid DNA samples using the PolYmerase chain reaction). In addition to reviewing the methods used by other authors, we also summarize our experience with deletion mapping of Y-chromosome in dioecious model plant species (*Silene latifolia*).

Keywords: physical maps; radiation hybrid mapping; recombination; deletion; HAPPY mapping; RH mapping software

Where and why to use the recombination independent mapping?

Genome maps are used for comparative phylogenetic studies, map-based cloning and marker-assisted molecular breeding. So far, the type of genetic maps that measure distances between loci using recombination frequencies, usually called "linkage maps", has been widely used. The advantage of these maps is that they are relatively easy to obtain, and they enable simultaneous mapping of phenotypic and molecular markers. However, there are also factors complicating the construction of this kind of maps. Variability in the crossing-over frequency along the chromosomes is a phenomenon generally occurring in higher organisms (Korol et al. 1994). Sometimes, a reduced rate of recombination can cause serious problems in recombination mapping; either of some parts of chromosomes, as it was observed in *Zea mays* (discussed e.g. by Kynast et al. 2004), or even of whole chromosomes (see the example of non-recombining regions of heterochromosomes of mammals or in dioecious species, reviewed in Vyskot and Hobza 2004). In some cases, the problem of non-recombining regions is avoided by means of construction of physical maps based on arrangement of large cloned fragments of the studied genome. When the non-recombining region is relatively small, it is possible to perform its physical mapping; this is currently being performed in *Carica papaya* by the team of Professor R. Ming, Laboratory of Plant Biology, University of Illinois at Champaign-Urbana (personal communication). However, construction of such maps is much more difficult in comparison with recombination mapping; moreover, in the case of species with large genomes it needs some previous mapping data. A typical example is the physical mapping of the non-recombining region of human Y-chromosome. This task started with a preparation of the initial map using the methods called "radiation hybrid mapping", together with FISH techniques; this map served as a base for assembling contigs of BACs. It continued by complex special methods, such as genomic clone subtraction and dissection of sequence family variants; both methods serve to overcome problems with repetitive sequences

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(Tilford et al. 2001). A more detailed description of the radiation hybrid method is given below.

Radiation hybrid mapping variants in animals and plants

Radiation hybrid (RH) mapping is a method based on the fact that after irradiation the probability of the simultaneous rescue of two markers is proportional to their proximity on the chromosome (Goss and Harris 1975). The panel of radiation hybrid cell lines is usually constructed by fusion of irradiated diploid human fibroblasts with hamster cells. Whole-genome RH panels were constructed and characterized for a variety of mammalian species including human, pig, cow, rat, dog, cat and mouse (reviewed in Himmelbauer et al 2000).

In spite of the progress in the last five years, the methodology of the radiation hybrid mapping in plants has not been developed as much as in animal models. The technique of RH mapping was applied only to a limited amount of species and the maps are not as detailed as in the case of mammals. Basically, three possible approaches exist:

(1) The approach of Wardrop et al. (2002) used for the construction of radiation hybrid panels for mapping in barley is the most similar to the protocols used in mammals. Their method is based on the fusion of the irradiated barley protoplasts with the protoplasts of tobacco, and subsequent cultivation of calli coming from individual fused protoplasts.

(2) Even better results were obtained using the approach based on "the radiation hybrid lines" obtained from crossing addition lines of oat (*Avena sativa*) and maize (*Zea mays*), using the embryo rescue technique. In this kind of experiment, addition lines are subjected to irradiation and the remaining procedure is the same as in the study of true radiation hybrids (Kynast et al. 2004). Similar approach was also used in *Gossypium* (Gao et al. 2006) and in wheat 1D chromosome (Kalavacharla et al. 2006).

(3) New possibilities are related to the usage of the "radiation hybrid" approach in the study of large non-recombining regions in sex chromosomes (Y or W). In this case, it is not necessary to perform inter-specific hybridisation, usual in distinguishing Y chromosome-linked copies from the X-linked ones, and to design sex specific primers. The only dioecious plant species where this approach has been applied is *Silene latifolia* (e.g. Lebel-Hardenack et al. 2002). The main reason why researchers prefer this model species is the fact that *Silene latifolia* has well distinguished pairs of sex chromosomes (Y being larger) that enables to combine cytological and molecular data, and a long tradition of the studies of sex determination in this species (reviewed in Vyskot and Hobza 2004). However, this method can be very useful also for other species with a large sex-specific non-recombining region.

Deletion mapping of the Y chromosome in plants – experiments

The series of deletion mutants are usually induced by irradiation of pollen using either X-rays (Lebel-Hardenack et al. 2002) or gamma irradiation (Barbacar et al. 1997, Zluvova et al. 2007); the latter is subsequently used for direct pollination. The experiments presuppose that deletions of markers on autosomes and on the X chromosome will be masked by their wild-type variants coming from the maternal genome, whereas deletions of Y-specific markers are detectable because the Y chromosome is present only in one copy. The most commonly used type of markers is represented by PCR-based ones (e.g. STS, EST or AFLP). Contrary to the genetic mapping, relatively a high number of markers is necessary. If the number of markers is low, the obtained map may not correspond to the true marker order. For example, doubling of the number of informative markers led to a higher statistical support and change of marker order on the *Silene latifolia* Y chromosome map (Zluvova et al. 2005).

Statistical evaluation of the deletion mapping data

The frequency at which markers are co-retained or co-deleted determines not only the order of markers (Goss and Harris 1975), but also relative distance between markers in centiRads. The parameters for the probability of breakage and the probability of retention depend on a given radiation dose. It means that when data from different experiments are combined, only the orders of markers should be counted, not the distances.

It is necessary to stress that it is impossible to evaluate data correctly without appropriate computer software; it is because of a huge amount of data obtained from one mapping experiment, as well as a possible occurrence of multiple deletions within one mutant. Fortunately, there are

many programs freely available. One of the oldest programs, but still often used, is RHMAP package (Boehnke et al. 1991), which is relatively fast and runs on the Windows platform. Program can be downloaded from the following address: http://csg.sph.umich.edu/boehnke/rhmap.php. Its only disadvantage is the interface that is not intuitive and user-friendly. Relatively recently, a new, much more user-friendly program CARHTA GENE, was published (de Givry et al. 2005). The program is available on www.inra.fr/bia/T/CarthaGene, and is gratis. Numerous RH mapping programs are listed by Ben-Dor et al. (2000), and another comprehensive list of RH mapping programs is available on http://compgen.rutgers.edu/rhmap/.

The first step in an analysis is to predict the logarithm of the odds of linkage (LOD) for every possible marker pair. A marker pair has a LOD = 1 when a chance of linkage is 10:1. A LOD score of at least 3 (i.e. a chance of linkage is 1.000:1) is generally accepted as an indicator that two sequences are linked. Another method how to test linkage is represented by the Fisher Exact Test. The program for the computation of the Fisher Exact Test is available on the web page: www.physics.csbsju.edu/stats/exact.html. According to the analysis, every group of markers showing absolute linkage (i.e. LOD is infinite) is reduced to one representative, and markers showing no linkage (i.e. LOD is zero) are omitted.

The simplest algorithm used is based on minimum obligate breaks criterion and it calculates the minimal number of chromosome breaks required to account for the observed deletions in the mutant chromosomes. This method has no special computational requirements. However, although it (intuitively) seems to be perfect, it is not always the case (reviewed in Ben-Dor et al. 2000). Other algorithms (e.g. maximum likelihood) are much better in this respect, but they often require prior knowledge of the system analysed. In principle, by using these methods it is possible to compare all marker orders and to choose the order with the highest probability. Yet, this approach is extremely slow. To significantly decrease the computational time, different algorithms for the solution of the travelling salesman problem are used (reviewed in Agarwala et al. 2000).

Conclusions and perspectives

Recombination-independent mapping methods represent not only an important part of the research in new model species, but they also enable to complete mapping in relatively well studied species. In the near future, we can expect a broader usage of these methods in plant genetics and breeding. Refining of existing maps should create better conditions for cloning of agriculturally important genes. Detailed comparative studies of extremely large non-recombining regions (such as some Y-chromosomes) should bring new insight to the evolutionary mechanisms leading to recombination arrest.

A very interesting alternative for the construction of platform for the physical mapping projects is a strategy called HAPPY mapping (mapping based on the analysis of approximately HAPloid DNA samples using the PolYmerase chain reaction); it is a tool to make accurate maps of genomes devised by Dear and Cook (1993). Its principle is similar to the radiation hybrid mapping; the difference is that the subjects of breakage are isolated DNA molecules. Afterwards, dilutions of the sample are prepared so that numerous samples are produced containing just one copy of the studied region. The subsequent experimental and statistical treatment is similar to the radiation hybrid mapping. This approach was applied with success in human, *Dictyostelium*, *Tetrahymena*, and *Arabidopsis* (reviewed in Waugh et al. 2002). The only limitation seems to be the necessity of a high number of markers for the studied region.

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